The Swedish Journal of Scientific Research ISSN: 2001-9211.Vol. 2. Issue 3. March. 2015

THE EFFECT OF SPORT DIVING ON STEM CELLS CD^{34+} AND COMPLETE BLOOD PICTURE

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Abstract

The purpose of this study was to assess the effect of sport diving on stem cells CD^{34+} and complete blood picture.

Materials and Methods: 10 male sport divers aged (23-34 yrs) with a history of diving of (5-1o years) were recruited for this study. They were instructed to dive for 45 min. Blood sample was drawn before and after diving in Hurghada Area, complete blood picture was estimated using coulter counter and CD^{34+} was determined by flow cytometry.

Results showed an increased CD^{34+} after diving compared to rest state. Also, complete blood picture increased as well.

Conclusion: The increased CD^{34+} counts might indicate that diving is involved in the wellbeing and health of divers.

The elevated blood picture reported an improvement in fitness and immunity and deserve further study in diving based rehabilitation programs.

KEYWORDS: Diving. CD^{34+} stem cells. Complete blood picture.

1. INTRODUCTION

Both commercial and sport diving expose divers to stresses that are unique to the "underwater environment". That is, terrestrial counter parts of the stresses do not exist, or they are ordinary so minimal that they go unnoticed while a person is on land. Three main categories of stresses are encountered in the underwater environment: physical, physiological, and psychological. In almost all circumstances the diver resolves the stresses of the underwater environment through positive feedback mechanisms *(Strauss and Aksenov, 2004).*

The human diver's responses to the underwater environment are of several types: physiological, automatic adjustments that the diver's body makes these improve performance and do not harm the diver. The elevation of heart rate with exercise is an example of the physiological response to activity.

Acclimatizations, temporary changes that improve performance and occur as a result of practice or continuing exposure to the activity. Adaptations, permanent changes that improve performance. These changes are inherited Bozanic (2002). He also added that these responses are associated with positive feedback. They help the diver to better perform in the underwater environment in different perspectives: cardiovascular, respiratory, blood and muscle tissue, thermal, propulsion and orientation. Espersen et al., (2002) reported that blood is a complex mixture of cells, fluids and substances dissolved in the fluids. RbCs carry 97.5% of oxygen in the blood stream in their hemoglobin, which is an iron-containing molecule found in red blood cells that binds oxygen in the presence of high oxygen concentrations and releases it when surrounded by tissues with lower oxygen concentrations. Echenhoff and Hughes, (1984) reported an elevation of blood cells with repetitive diving.

Identification of EPCs on the cell surface expressions of various protein markers. There is no straight forward definition of an EPC marker because these cells seem to be a heterogeneous group associated with different cell surface antigen expression profiles. The most commonly described molecules that serve as biomarkers for recognition of an EPC population include CD^{34+}, and CD^{133}. The pioneer study of Asahara et al., (1997) recognized EPCs as CD^{34+} mononuclear cells.

CD^{34+} cells are multipotent progenitors that can engraft in several tissues (Krause et al., 2001), circulating CD^{34+}cells can be used to indirectly estimate hematopoiesis based on CD^{38}, human leukocyte antigen and CD^{33} markers (Terstappen et al., 1997). In humans, both acute exercise (Rehman et al., 2004) and training (Laufs et al., 2004) have been shown to mobilize endothelial precursors, but the physiological response to hematopoietic cells to exercise has been systematically assessed.

The aim of this study is to reveal the effect of diving on stem cell CD^{34+} and complete blood picture.

The Questions to be solved:

1. What is the role played by diving on stem cell determination?
2. What is the role played by diving on complete blood picture?

2. MATERIAL AND METHODS

Ten healthy male divers aged (23-34 years), with a history of diving of (5-10 years) were recruited for this study. They were instructed to dive for 45 min. All participants were screened and asked to fill out healthy history, they were non diabetic, and free of cardiovascular, lung, diver disease. Participants did not take any medications that affect EPCs number or function. These include ACE inhibitors, statins and angiotensin II receptor agonists.

All participants refrained from caffeine and any medications or vitamins 48 hours prior to the test. Blood sample was drawn before and after the diving heart rate and BMI together with stem cell $CD^{34+}$and complete blood picture (Hb, Rbcs, Wbcs, MCV, MCH, MCHC, hematocrit and platelets), complete blood picture was determined by coulter counter and stem cell CD^{34+} by flow cytometer.

In case of CD^{34+} stem cell, the fluorochrome conjugated antibody was used to permit the identification and numeration of cell populations expressing the CD^{34+} antigen present in human biological samples using flow cytometry.

Table 1: Basic characteristics

Variables	M	S.D	Ske.
Age (years)	28.1	2.6	-2.3
Height (cm)	172.75	4.30	0.52
Weight (kg)	72.30	11.68	-0.12
Pulse rate (Count/min)	64.6	8.9	0.73
BMI	23.55	5.31	0.44

Skew factor for participants were between (±3) values are means ± SD, P < 0.05.

Blood sample was drawn by a medical specialist at 19-21/12/2012 complete blood picture and CD^{34+} stem were determined in a specialized laboratory. Diving process occurred in Hurghada area.

Tools and devices used:

1. Scuba apparatus.
2. Syringes, cotton, alcohol.
3. Test tubes.
4. Balance weight, Restameter.
5. Freezer and Coleman.
6. EDTA anticoagulant.
7. Coulter counter.
8. Centrifuge.
9. Flow cytometer.
10. Monoclonal antibodies against CD^{34+}.
11. Fluorochrome.
12. Vortex.
13. Pulse meter.
14. Ice box.

The type of Scuba apparatus used in more than 99% of all sports and commercial diving is the open circuit demand system. This system consists of the following component (1) one of more tanks of compressed air or breathing mixture. (2) a first stage reducing valve for reducing the pressure. (3) a combination inhalation demand valve and exhalation valve that allows air to be pulled into the lungs with slight negative pressure of breathing and then to be exhaled into the sea at a pressure level positive to the surrounding water pressure (4) a mask and tube system with small dead space ***(Graver, 1999).***

Phase of a dive

All diving activities can be divided into four phases:

- Surface phase.
- Descent phase.
- Bottom phase.
- Ascent and post-dive.

The surface phase of the dive includes all activities from donning gear until the diver is ready to start the descent. The descent phases commences when the diver leaves the surface. The bottom phase reflects the time from competent the descent to the time of leaving the bottom. Most sports Scuba divers make the ascent with stops at several depths as in the profile 60 FSW (18 MSW) for 30 minutes, 45 FSW (14 MSW) for 20 minutes, and 25 FSW (7.6 MSW) for 10 minutes. It is now recommended that all sport scuba divers perform a rest stop at 15 FSW (4.6 MSW) during the ascent before surfacing. Exponential efforts and energy requirements are quite different for each phase. The surface phase is usually the most challenging because of the effort of carrying the diving gear to the diving site, donning the gear, and entering the water. The main challenge of the descent phase of the dive is the equilibration of pressure in the middle ear spaces (Berger, 2000).

Statistical Analysis: Student "t" tests were used to test the differences before and after diving, the difference was tested using a measure of analysis of variance (ANOVA). An α level of 0.05 was used to indicate statistical significance.

3. RESULTS

Ten sport divers participated in the study. They were matched for age, weight, height, pulse rate and BMI non-significant changes were noticed in basic characteristics (Table 1).

Hematological values in pre and post diving was recorded for Rbcs, Wbcs, HB, MCV, MCHC, Hematocrit, MCH, and platelets (Table 2). The results revealed a significant elevation in all parameters after sport diving. Data for CD^{34+} number and percent were recorded in Table (3), revealed a significant increase in both CD^{34+}counts and percent for the sake of post diving compared to the rest state.

Table 2: statistical differences of blood cells between
pre and post diving (n = 10)

Variables	Pre Diving	Post Diving	Significance
Hemoglobin (gm/dl)	13.4 ± 1.2	15.6 ± 1.5	S
Rbcs (mil/cmm)	4.6 ± 0.8	5.9 ± 0.6	S
MCH (pg)	27.9 ± 1.1	29.7 ± 1.3	S
Hematocrit %	39.7 ± 1.5	43.9 ± 1.6	S
MCV (FL)	84.1 ± 0.5	86.8 ± 0.4	S
MCHC %	42.4 ± 1.7	45.6 ± 0.9	S
Wbcs (Thou/cmm)	7.8 ± 0.7	9.2 ± 0.6	S
Platelets (Thou/cmm)	230.6 ± 3.5	264.7 ± 4.1	S

Table (2) indicated a significant difference between pre and post diving $P < 0.05$ Mean ± SD

Table 3: statistical differences of $CD34^+$ counts and $CD34^+$% between
pre and post diving (n = 10)

Variables	Pre Diving	Post Diving	Significance
CD^{34+} (counts)	56.4 ± 3.4	132.2 ± 5.2	S
CD^{34+} (%)	0.93 ± 0.1	3.4 ± 0.6	S

Table (3) indicated a significant difference between pre and post diving in CD^{34+}counts and % $P < 0.05$

4. DISCUSSION

Physiological stresses are associated with the normal functions of the body. Physiological stresses associated with diving relate primarily to ventilation, that is, the breathing in the oxygen and the exhalation of carbon dioxide. In addition, nitrogen, the inert gas that comprises approximately 79% of the air we breathe, equilibrates with the nitrogen in our body tissues. Two methods mediate the ventilation stresses on a diver while underwater: breath holding and the supply of an air source to the diver (Bachrach and Egstrom, 1999).

The data presented in Table (2) revealed a significant increase in (Hb, Rbcs, Hematocrit, MCV, MCH, MCHC) after diving compared to the resting state.

Barrett et al., (2010) in Ganong review of medical physiology stated that blood consists of a protein rich fluid known as plasma, in which are suspended cellular elements: Rbcs, Wbcs and platelets, which are formed in the bone marrow which is actually one of the largest organ in the body. Hematopoietic stem cells are bone marrow cells that are capable of producing all types of blood cells.

Burge et al., (1993) and Gillen et al., (1991) reported that acute effect of exercise on blood is to cause release of fluid from the vascular component, which decreases the volume of plasma and blood. This decreases volume cause hematocrit and cellular element to increase, which is termed hemoconcentration. The hemoconcentration may be the main cause of the increased blood cells.

Robergs and Roberts (1997) stated that the main functions of the cellular components of blood are the transport of oxygen and carbon dioxide, blood clotting, acid base buffering, immune functions and tissue repair and destruction, heat transfer and thermoregulation, and plasma help water exchange and transport, circulation of hormones, circulation of metabolites, and waste products.

Hemoglobin bind oxygen in the lungs, then the oxygen laden red blood cells are carried by the blood stream throughout the body to the tissues. The tissues then utilize the oxygen for metabolic purposes. All cells of the body require oxygen such as the brain, the muscles and the heart and it is not by coincidence that the diving reflex ensures adequate provision of oxygen to the most critical tissues of the body during the breath hold dive (Marabotti et al., 1999 and Castellini et al., 2001).

Guyton and Hall (2006) reported that white blood cells are the mobile units of the body's protective system. They are formed partially in the bone marrow (granulocytes, monocytes and a few lymphocytes) and partially in the lymph tissue (lymphocytes and plasma cells). They provide a rapid and potent defense against infectious agents. They also added that the adult human being has about 7000 white blood cells per microliter of blood and that the number of platelets, which are only cell fragments in each microliter of blood is normally about 300000.

The increased Wbcs and platelets in Table (2) revealed that these cells work together to prevent disease by destroyed invading bacteria and forming antibodies and that platelets is an active structure that help in the blood-clotting process.

The data presented in Table (3) revealed a significant increased CD^{34+} stem cells count and percent after diving. Many authors reported an increased stem cells concentration after exercise Zaldivar et al., (2007), Jung et al., (2008), Hoellriegel et al., (2007), Hoellriegel et al., (2008) and Laura Bilek (2008), Wall et al., (2008).

The number of circulating stem cells represents the balance between liberation of stem cells from the bone marrow and incorporation at the level of the vessel. Laufs et al., (2005) demonstrated that CD^{34+}increased after 30 minutes of high intensity running in healthy participants.

Ewa and Parvet (2007) reported that a decrease in the blood supply to a bodily organ or tissue, caused by constrictor or obstruction of the blood vessels is a common cause of ischemia.

Adams et al., (2004) found that peripheral blood stem cells counts were increased significantly in ischemic patients within 24-48 hours after exercise, this was accompanied with elevation of VEGF concentration in plasma. These results confirmed that VEGF is a significant factor responsible for stem cells stimulation from bone marrow to peripheral blood.

Sarah Witkowski, (2010) reported that exercise may improve the number and function of stem cells while improving oxidative stress status.

The researchers' opinion is that stress and ischemia might be the main factors in stimulating stem cells and blood cells egress.

The discussion answer the questions to be solved, about the role played by diving on stem cells determination and about the role played by diving on complete blood picture. Diving play a positive role in increasing both stem cells and cellular blood components.

5. CONCLUSION

- CD^{34+}counts increased after diving due to stress, indicating a positive effect in well being and health of participants.
- The increased blood cells counts indicated an improvement in fitness and immunity, and deserve further study in diving based rehabilitation programs.

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